REMARKS

Applicants respectfully request that the foregoing amendments be made prior to examination of the present application.

I. Specification Amendment

The specification has been amended at page 37, last full paragraph, to correct the typographical error contained at position 218 in the amino acid sequence of SEQ ID NO:142, specifically by deletion of (I) Isoleucine and insertion of Threonine (T). The specification amendment is supported in SEQ ID NO:142 of the Sequence Listing filed June 23, 2006.

II. Status of Claims

A preliminary amendment filed February 26, 2007, effected cancellation of claims 1-94, without prejudice or disclaimer. By the present preliminary amendment, Applicants seek cancellation of claims 95-136 as well, likewise without prejudice or disclaimer, and the addition of claims 137-139. Upon entry of this amendment, therefore, claims 137-139 will be pending.

III. Support for the Claims

As discussed in greater detail below, the present claims are supported in the specification at page 35, line 5 – page 38, last full paragraph, and in Examples 15, 16, and 19. Thus, the antibody of the present claims has two heavy chains and two light chains, in keeping with "the fundamental structure of immunoglobulin" proteins, detailed in the specification at page 38, lines 17 and 18. Accordingly, the present amendment introduces no impermissible new matter.

IV. Patentability of the Claims

The antibody of the present claims is designated "4D11G4PE" in Applicants' specification (see page 35, line 7 from the bottom, for instance) and is a mutant of an anti CD40 antagonistic antibody, designated "4D11," which is described in a published PCT application, WO 02/088186 (see the specification, *e.g.*, in the initial two paragraphs on page

5, and the information disclosure statement filed June 23, 2006). More particularly, the claimed antibody is an anti-CD40 antibody produced by hybridoma 4D11 (Accession No. FERM BP-7758), in which the constant region of heavy chain is IgG4 and a serine at position 228 is substituted with proline (S228P) and leucine at position 235 is substituted with glutamate (L235E). Thus, in the antibody designation "G4" denotes a subclass, "P" denotes S228P and "E" denotes L235E.

By way of some background, CD40 is an antigen that is expressed on B cells and dendritic cells. CD40 interacts with a ligand therefore, CD40L, which is expressed on CD4+T cells. Dendritic cells are activated when CD40 expressed on dendritic cells interacts with CD40L on CD4+T cells. The activation of dendritic cells enhances the expression of auxiliary stimulants, such as CD80 and CD86, and the production of IL-12, which results in induction of cellular immunity by cytotoxic T lymphocyte. Furthermore, when CD40 on B cells interacts with CD40L on CD4+ T cells, B cells grow and differentiate, and antibody production by the B cells is enhanced, resulting in induction of humoral immunity.

An antagonistic anti-CD40 antibody binds to CD40 on B cells and dendritic cells, respectively, to block the interaction between CD40 and CD40L on CD4+ T cells, which in turn inhibits the induction of cell immunity and humoral immunity. Such an antibody is expected to be useful as a suppressor of rejection, following organ transplantation, or as a drug for treating autoimmune disease.

Yet, when an antibody that recognizes antigens on cells involved in immune responses, such as B cells and dendritic cells, binds to an antigen on such cells, then the Fc region of the antibody interacts with Fc receptors presented by other cells, which induces signals for an immune response in some cases. Therefore, when an antibody such as an antagonistic anti-CD40 antibody is used to suppress rejection after organ transplantation or to treat autoimmune disease, "it is important that anti-CD40 antagonistic antibodies have no activity to induce signals by their *in vivo* crosslinking via Fc receptors, even if the ADCC activity cannot be detected" (the specification at page 27, last full sentence). This is so because, if such antibodies "induce an agonistic activity due to some effect after they are

administered to patients, however weak [the activity] may be, [then] the symptoms may worsen in contrast to the desired therapeutic effect" (page 28, second full sentence).

Accordingly, for an antagonistic anti-CD40 antibody it is very important to avoid the induction of CD40 signals (*i.e.*, agonistic activity) brought about by the crosslinking of the antibody via Fc receptors *in vivo*. Recognizing this goal but uncertain of its attainment, the present inventors prepared mutants of 4D11, the antagonistic anti-CD40 antibody 4D11 mentioned above, and eventually discovered the claimed antibody, 4D11G4PE, which does not exhibit the agonistic activity not only *in vitro* but also *in vivo*. Thus, as Applicants' Examples 15, 16, and 19 show, the antibody of the present claims does not enhance the production of IL-12 by the agonistic activity *in vivo* (Example 15) and has significant treating effects on a delayed hypersensitivity model (Example 16) and a skin graft model (Example 19).

So remarkable are these properties of the claimed antibody that it now is under evaluation in a clinical trial designed to evaluate its suitability as a drug. The unexpected properties of the claimed antibody likewise distinguish the latter over the relevant pre-filing literature, including those documents that Applicants' listed in the aforementioned information disclosure statement.

A number of those listed documents were cited in relation to a Japanese counterpart application, the claims of which substantially track the language of the present claims. Moreover, the Japanese Patent Office allowed the counterpart claims, in view of the prior art.

It is useful to note, therefore, that cited WO 02/088186 (information disclosure statement filed June 23, 2006) discloses an anti-CD40 antibody and, in that context, the amino acid and nucleotide sequences of the variable region of 4D11G4PE (SEQ ID NOs:47 to 50, respectively).

Cited WO 03/33538 (information disclosure statement filed December 11, 2008) discloses an anti HLA-DR antibody. More specifically, WO 03/33538 states that conversion of subclass to "IgG2 or IgG4" can modify the biding activity to Fc receptor, as can as mutations of the constant-region amino acid sequence.

Cited Japanese Patent No. JP-3101690 (information disclosure statement filed December 11, 2008) describes that amino acid mutations at positions 234, 235, 236, 237, 297, 318, 320 or 322 modify CDC or ADCC activity. Specifically, it discloses (i) mutation of amino acid at position 235 to Glu, (ii) mutation of amino acid at position 234, 236 or 237 to Ala, (iii) mutation of amino acid at position 318 to Ala or Val, (iv) mutation of amino acid at position 322 to Ala or Gln, and (v) mutation of amino acid at position 320 or 297 to Ala.

Cited *Brekke et al, Eur. J. Immunol.*, 24:2542-2547 (1994), (information disclosure statement filed December 11, 2008) describes modification of CDC or ADCC activity by virtue of (i) substitutions of Gln at position 268 to His, (ii) Ser at position 331 to Pro, and (iii) Gln at position 268 to His and Ser at position 331 to Pro, respectively (see "Abstract"). In general term, *Brekke et al.* explain the change of effector function by mutation of amino acids in the constant region of an antibody. There is no mention, however, of mutation at position 235.

The teachings of cited *Idusogie et al., J. Immunol.*, 164(8): 4178-4184 (2000), (information disclosure statement filed June 23, 2006) relate to an anti CD20 antibody, Rituxan®, in whch substitution of D270, K322, P329 or P331 in the constant region of IgG1 with Ala reduces the binding activity to C1q, thereby reducing CDC activity. *Idusogie et al.* explain in general terms the change of effector function by mutation in the antibody constant region, but it does not disclose mutation at position 235.

Cited *Angal, et al., Mol. Immunol.*, 30(1): 105-108, (1993), (information disclosure statement filed June 23, 2006) describes the substitution of Ser with Pro at position 241 of human IgG4, stabilizing the S-S bonding between the heavy chains. There is no suggestion whereby affected position 241, highlighted by *Angal, et al.* could be equated with position 228 in 4D11G4PE.

These references purport to describe only the reduction of ADCC activity and the like *in vitro*. Accordingly, they provide no guidance on how or even whether agonistic activity might avoided not only *in vitro* but also *in vivo*. This is significant because, while each mutation characterizing the claimed antibody, 4D11G4PE, is disclosed separately somewhere

in the cited references, the prior art as a whole would have given the skilled artisan no hint that combining the variable region of 4D11 (WO 02/88186) with each mutation as presently recited might result in an absence of agonistic activity *in vivo* as well as *in vitro*.

It is generally known, for example, that mutation of a single amino acid of an antibody can alter the structure and, hence, the features of the antibody. When plural mutations are combined, therefore, one mutation may negate the effects of the other mutations. Even if the effect of each mutation were known, therefore, the person of ordinary of skill could not have predicted that the combined mutations bring all effects of each mutation.

Furthermore, WO 03/33538 describes that IgG 2 and IgG 4 bring about the same effect. Japanese Patent No. JP-3101690 and Brekke *et al.* show many mutations other than a mutation at position 235 to Gln. All cited references disclose the *in vitro* reduction of ADCC activity and the like, but no reference suggests how to avoid agonistic activity *in vivo*. With such background information, the skilled artisan could not have entertained any reasonable *a priori* expectation of selecting a mutation, from among many disclosed in the art, such that signal induction by virtue of agonistic activity is avoided both *in vitro* and *in vivo*.

Surprisingly, however, the present inventors discovered that the claimed antibody, does not induce an increase in IL-12 production, an indicator of agonistic activity *in vivo*, that is, activation of CD40-expressing cells, such as monocytes, when an anti CD40 antibody binds thereto.* See Example 15 and Figure 16 of this application. Conversely, the inventors demonstrated that that an antibody (4D11G4P), the constant region of which was converted to IgG4 and Ser at position 228 of the heavy chain constant region, ***did*** induce increased IL-12 production (*id.*). Thus, the difference of a single amino acid of an antibody was shown to affect agonistic activity *in vivo*, underscoring the importance in this context of how one mutates the Fc region of the antibody.

This insight was the inventors' alone, since none of the references discloses mutation of the Fc region as presently recited. Applicants also would emphasize that advantages or

* For example, see Subauste, *J. Infectious Disease* 185 (Supp. 11): S83-89 (2002) (copy appended), who documented IL-12 production occasioned by binding of CD40 on dendritic cells by CD40 ligand on T cells.

counterproductive effects occasioned by the *in vivo* administration of a drug often cannot be predicted from *in vitro* studies, and that certainly is the case for *in vivo* reduction of agonistic activity of an antibody. In fact, although CDC activity of 4D11G4P antibody (as distinguished from the claimed antibody, 4D11G4PE) was reduced *in vitro*, as shown in Applicants' Example 12 and Figure 10, administration of the antibody to a cynomolgus monkey *in vivo* did induce IL-12 production (see discussion above of Example 15 and Figure 16).

Furthermore, there is an anti-CD4 antibody-related reference of record that actually teaches away the claimed inventions of the present application. Reddy *et al.*, *J. Immunology* 164: 1925-33 (2000), (cited in information disclosure statement filed June 23, 2006), discloses two mutated versions, kelisimab (IgG1) and clenoliximab (IgG4PE), of an anti-CD4 antibody. Clenoliximab, a mutant of kelisimab, incorporates the heavy chain constant region of IgG4 and embodies two amino acid substitutions, S228P (serine to proline at position 228) and L235E (leucine to gluatamate at position 235).

The subclass and the mutations of the disclosed molecules track those of the presently claimed antibody. Yet, Reddy *et al.* teach that clenoliximab loses the binding activity to Fc receptor *in vitro* (Figure 4B), which reduces the induction of CD4 modulation *in vitro* (Figure 8A). CD4 modulation is caused by (1) internalization of CD4 on T cells (*i.e.*, uptake of receptors into a cell) or (2) "stripping" or digestion of extracellular portion of a receptor) of CD4 on T cells. As result of CD4 modulation, the number of CD4 on T cells decreases, to wit: "CD4 mAbs can induce modulation of CD4 on the surface of T cells ...." (page 1929, left column, lines 16 to 18). It is predicted that CD4 modulation takes place by the induction of CD4 signaling (*i.e*,. agonistic activity) caused by crosslinking of anti-CD4 antibodies via Fc receptors. On the contrary, however, Reddy *et al.* state that clenoliximab induces strong CD4 modulation *in vivo*, unlike the *in vitro* result: "clenoliximab causes strong CD4 receptor modulation in animal models ..." (page 1932, left column, in lines 27 *et seq.*).

Reddy *et al.* surmised (i) up-regulation of Fc receptors under inflammatory disease conditions *in vivo*, (ii) higher Fc receptor levels on tissue-resident macrophages than on non-stimulated, circulating blood monocytes, and (iii) the impact of factors that can effect cross-

linking of antibodies bound on cell surfaces (see page 1932, right column, at lines 5 - 9). In any event, the Reddy publication confirmed that clenoliximab induces CD4 modulation under the stimulation of monocytes by Fc receptors that are highly expressed on monocytes.

The antibody of the claimed invention is an anti-CD40 antibody, which is different from the anti-CD4 antibody of Reddy *et al.* Nevertheless, an anti-CD40 antibody is similar to an anti-CD4 antibody in that both act on immune cells and in that induction of signaling can occur by Fc receptor-mediated crosslinking of the antibodies, when the latter bind to a target antigen on cells. Furthermore, the inhibition of the agonistic activity *in vivo* is desirable for an anti-CD4 antibody and an anti-CD40 antibody alike, if they are to be used therapeutically, especially for treating immunological disease.

As noted above, Reddy *et al.* showed that clenoliximab effects strong CD4 modulation *in vivo* due to crosslinking of the antibody on CD4 via Fc receptors, which are highly expressed. As the present application teaches, such induction of agonistic activity *in vivo* must be avoided if the involved antibody is to find use as a drug.

For the presently claimed anti-CD40 antibody, accordingly, a skilled person informed by the Reddy publication would have expected the same phenomenon documented by Reddy *et al.* for clenoliximab, given the embodiment by both antibodies of the same amino acid mutation. By the same token, the therapeutically disadvantageous aspect of clenoliximab, discussed above, would have negated any motivation for the skilled person to have employed such mutation in an effort to produce an anti-CD40 antibody that did not induce agonistic activity *in vivo*.

CONCLUSION

For each of the reasons detailed, the present claims are patentable over the prior art, as illustrated by the publications of record. Moreover, claims 137 – 139 should be considered together. For example, see PCT Rule 13.2 (mandates unity-of-invention finding "when there is a technical relationship among those [claimed] inventions involving one or more of the same or corresponding special technical features"), MPEP § 806.05(c) (states that subcombination/combination restriction is improper if combination requires "particulars of

the subcombination as claimed for patentability"), and MPEP § 821.04(b) ("claim(s) directed to a product which [are] found allowable" should be grouped with "process claims which depend from or otherwise require all the limitations of an allowable product claim").

Applicants believe, therefore, that this application is in condition for allowance, and they request an early indication to this effect. Examiner Gambel also is invited to contact the undersigned directly, should he feel that any issue warrants further consideration.

Respectfully submitted,

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